



Bioelectric medicine: unveiling the therapeutic potential of micro-current stimulation

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Abstract

Bioelectric medicine (BEM) refers to the use of electrical signals to modulate the electrical activity of cells and tissues in the body for therapeutic purposes. In this review, we particularly focused on the microcurrent stimulation (MCS), because, this can take place at the cellular level with sub-sensory application unlike other stimuli. These extremely low-level currents mimic the body's natural electrical activity and are believed to promote various physiological processes. To date, MCS has limited use in the field of BEM with applications in several therapeutic purposes. However, recent studies provide hopeful signs that MCS is more scalable and widely applicable than what has been used so far. Therefore, this review delves into the landscape of MCS, shedding light on the multifaceted applications and untapped potential of MCS in the realm of healthcare. Particularly, we summarized the hierarchical mediation from cell to whole body responses by MCS including its physiological applications. Our final objective of this review is to contribute to the growing body of literature that unveils the captivating potential of BEM, with MCS poised at the intersection of technological innovation and the intricacies of the human body.

Keywords Microcurrent stimulation · Therapeutic effects · Hair growth · Skin inflammation · Lipolysis · Non-alcoholic fatty liver diseases (NAFLD)

1 Introduction

Bioelectric medicine (BEM) refers to the use of electrical signals to modulate the electrical activity of cells and tissues in the body for therapeutic purposes. The human body, with its intricate network of cells and tissues, relies extensively on bioelectricity for communication and regulation [1]. BEM seeks to harness these natural electrical phenomena to treat and regulate various conditions.

Historically, it was not until the late sixteenth century that a true science of bioelectricity was born. This was the discovery of ‘animal electricity’ by Luigi Galvani [2, 3]. In the centuries since the discovery of animal electricity, much has been learned about how electricity is involved in the activities of all cells and tissues, and organs throughout

the body. In the middle of the 1900s, researchers began a series of important and controversial studies of the role of electricity in development and disease [3, 4]. From the work on the development of the nervous system, they realized how little was actually known about the control of form in animals and the human body. Molecular genetics was revealing how the parts of the body are manufactured, however, there was little understanding that directs their assembly into the whole organism. While there continues to be widespread enthusiasm about the discoveries being made in molecular biology, the mystery of the physiological mechanism continues to this day. This is a very important issue. All healing must reference the physiological mechanism of the body so that the repair process recreates as closely as possible the form that was present before an injury or disease. That is the reason why we living in the current generation must pay attention to BEM.

In recent years, the convergence of biology and technology has given rise to an exciting and rapidly evolving field known as the aforementioned BEM. The current BEM is an interdisciplinary field that combines principles of medicine, biology, and engineering to understand and utilize electrical signaling in the body [5].

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The physiological effects on the human body via extrinsic stimulations are often discussed in terms of distinct levels of increasing complexity, from the smallest chemical building blocks to a unique human organism. As is well known, a cell is the smallest independently functioning unit of a living organism. Humans are multicellular organisms with independent cells working in concert together. All living structures of human anatomy contain cells, and almost all functions of human physiology are performed in cells or initiated by cells. Meanwhile, a tissue is a group of many similar cells that work together to perform a specific function and further extend to the organ's function.

The most current of biological and medical research have provided unprecedented insights into the cellular processes which is valuable to use therapeutic purposes. Despite these insights, however, it is arguable that there is still only limited predictive understanding of cellular behaviors and relative physiological activities. In particular, the basis of molecular and cellular behavior and the initiation of many different metabolic, transcriptional, or mechanical responses to environmental stimuli remain largely unexplained. Therefore, to go beyond the current status, it is needed to understand the intracellular and extracellular conditions emerging from electrical stimulation.

The type of bioelectrical interventions can be broadly categorized by their characteristics and targeted applications including transcutaneous electrical nerve stimulation (TENS) [6, 7], electrical muscle stimulation (EMS) [8], functional electrical stimulation (FES) [9], and micro-current stimulation (MCS) [10, 11] (Table 1).

Among these, we particularly focused on the MCS in this review, because, it is known that MCS, unlike other stimuli, can take place at the cellular level with sub-sensory application. MCS involves the application of low-level electrical currents, typically in the range of microamperes (less than 1 mA), to specific areas of the body for therapeutic purposes [12, 13]. These extremely low-level currents mimic the body's natural electrical activity and are believed to promote various physiological processes. MCS aims to influence cellular activity, promoting cell repair and regeneration. It is believed to enhance cellular energy such as ATP production and stimulate the healing process at a cellular level [13, 14]. Whereas, TENS, EMS, and FES utilize higher-level electrical currents, typically measured in milliamperes (mA). These stimulate motor neurons, causing muscle contraction or neural activation at a tissue level [15]. These can be beneficial for muscle or nerve rehabilitation after injury and for improving muscle or nerve coordination [16, 17].

In summary, the cellular-level focus of MCS sets it apart from other techniques that primarily target muscles or nerves. While approaches like EMS, TENS, and FES stimulate muscles or nerves directly, MCS operates at

a more fundamental level, influencing cellular activity. MCS, with its low-level electrical currents typically measured in microamperes (μA), aims to modulate cellular functions, enhance ATP production, and promote cellular repair and regeneration. This cellular focus allows MCS to potentially address underlying issues related to tissue damage, inflammation, and cellular dysfunction. In contrast, techniques targeting muscles or nerves directly, such as EMS, TENS, and FES, often aim to induce muscle contractions or neural activation at the tissue level. While effective for specific purposes like muscle rehabilitation or pain management, these approaches may not delve as deeply into cellular-level processes. Fundamentally, the distinction lies in the depth of intervention. MCS's cellular focus may provide a unique avenue for addressing conditions at their foundational level, potentially contributing to more comprehensive therapeutic outcomes.

While MCS potentially contributes to more comprehensive therapeutic outcomes, its application in the field of BEM has been limited, primarily focusing on pain management [18], wound healing [19], and cosmetic treatments [20] to date. However, recent studies show promising signs that MCS is more scalable and widely applicable than previously explored.

This review, therefore, delves into the landscape of MCS, shedding light on the multifaceted applications and untapped potential of MCS in the realm of healthcare. Particularly, we summarized the hierarchical mediation from cell to whole body responses by MCS including its physiological applications. Our final objective of this review is to contribute to the growing body of literature that unveils the captivating potential of BEM, with MCS poised at the intersection of technological innovation and the intricacies of the human body.

2 Categorization of electrical stimulations

The categorization of electrical stimulation includes a wide array of therapeutic methods utilizing electrical currents to achieve specific physiological effects. These modalities serve various purposes, from pain management to muscle rehabilitation and cellular functional improvement. Therefore, it is necessary to understand the distinct categories to tailor interventions according to individual patient needs. Here are several categories.

TENS is a prominent modality designed for pain relief [21]. It involves the application of low-voltage electrical currents through skin electrodes, modulating nerve signals and alleviating the perception of pain. TENS finds widespread use in conditions such as chronic pain, arthritis, and musculoskeletal disorders.

Table 1 Categorization of electrical stimulations

Categorization	Purpose	Mechanism	Controllable Parameters	Reference
Transcutaneous Electrical Stimulation (TENS)	Primarily used for pain relief	Involves the use of low-voltage electrical currents to stimulate sensory nerves, inhibiting pain signals	Intensity (mA): adjustable to suit the individual's comfort level Frequency (Hz): adjustable between 1 to 200 Hz Pulse Width (ms): Ranges from 0.05 to 200 ms	[29–35]
Electrical Muscle Stimulation (EMS)	Aims to stimulate muscle contractions for strengthening and rehabilitation	Delivers electrical impulses to motor nerves, causing muscles to contract	Intensity (mA): adjustable to control the strength of muscle contractions Frequency (Hz): adjustable in the range of 1 to 100 Hz Pulse Width (μ s): ranges from 200 to 3,000 μ s	[8, 36–41]
Functional Electrical Stimulation (FES)	Facilitates functional movements by stimulating paralyzed or weakened muscles	Applies electrical currents to nerves controlling muscles to induce specific movements	Intensity (mA): variable based on individual needs and muscle response Frequency (Hz): adjustable in the range of 20 to 100 Hz Pulse Width (μ s): adjustable within the range of 50 to 600 μ s Stimulation Timing: precise timing to coincide with the desired movement	[42–48]
Microcurrent Stimulation (MCS)	Generally used for tissue repair, pain management, and enhancing cellular function	Involves extremely low-level electrical currents that mimic the body's natural electrical signals	Intensity (μ A): Typically ranges from 10 to 999 μ A Frequency (Hz): often in the sub-sensory range, around 0.1 to 1000 Hz Pulse Shape: can include square, triangular, or sinusoidal waves	[14, 49–55]

EMS, on the other hand, focuses on inducing muscle contractions [22]. By mimicking the body's natural muscle contractions through electrical impulses, EMS contributes to muscle strengthening, sports training, and post-surgery rehabilitation. This classification is particularly valuable for patients recovering from injuries or aiming to enhance their physical performance.

FES takes a unique approach, aiming to restore or improve functional movements in individuals with paralysis or muscle weakness [23]. By directly stimulating specific muscles, FES enables actions like walking or grasping, making it a crucial tool in neurological rehabilitation, especially after conditions like strokes.

One notable category is MCS, a technique that employs low-level electrical currents, typically measured in μA , closer to the body's natural electrical activity. Microcurrent stimulation has garnered attention for its potential applications in diverse medical fields. MCS operates on the principle that subtle electrical impulses, often sub-sensory, can influence cellular activity and promote healing. This technique is characterized by its ability to enhance ATP production, protein synthesis, and cellular regeneration. The relatively low intensity of the currents used in MCS sets it apart from other electrical stimulation modalities, making it well-tolerated and suitable for extended use [13, 24].

Essentially, the categorization of electrical stimulation modalities provides healthcare professionals with a nuanced understanding of each technique's purpose, mechanisms, and applications, enabling them to make informed decisions in tailoring treatment plans for diverse patient populations.

Meanwhile, electrical stimulation can be also categorized based on its intervention for specific therapeutic purposes [25]. The primary distinction between invasive, potentially involving bio-implant, and non-invasive electrical stimulation lies in their approach to delivering therapeutic interventions. Invasive electrical stimulation involves the direct placement of electrodes or devices inside the body, often requiring surgical procedures for implantation. This method allows for a high level of precision, enabling healthcare professionals to target specific neural structures or tissues with accuracy. Examples of invasive electrical stimulation include deep brain stimulation (DBS) for neurological disorders [26] and intramuscular stimulation (IMS) for precise muscle stimulation in rehabilitation [27, 28]. The invasiveness of these procedures, however, introduces potential risks associated with surgery and device implantation.

Conversely, non-invasive electrical stimulation does not necessitate the insertion of electrodes or devices into the body. Instead, it is applied externally, typically using electrodes placed on the skin surface. While non-invasive methods are generally less precise than invasive approaches, they are considered safer and more accessible.

The introduced stimulations including EMS, TENS, FES, and MCS are common examples of non-invasive electrical stimulation. These methods offer advantages in terms of reduced risks and increased patient comfort, as they avoid the complexities associated with surgical interventions.

Considerations surrounding the choice between invasive and non-invasive modalities often revolve around safety, precision, and accessibility. Non-invasive approaches are generally considered safer, with lower associated risks compared to invasive procedures, which may entail surgical complications and potential device-related issues. While invasive methods offer unparalleled precision by directly targeting specific tissues, the invasiveness of the procedures may limit their applicability, especially in cases where accessibility and convenience are crucial considerations.

3 MCS as a mediator for physiological changes in the molecular and cellular levels

3.1 General concepts

Electricity is one of the most significant forces of nature and has had a long history of involvement in BEM. While it was discussed as early stage that bioelectricity may be fundamental to understanding various cellular behaviors, the electrical investigations of cells were only focused on cellular bioenergetics [56, 57]. The bioelectrical view of cells as a more general concept has remained confinement to the fringes of biological research for several decades, during which molecular biology has made astonishing advancements in our understanding of cells and our ability to manipulate genes. Fortunately, the findings from molecular studies highlight once again the importance of bioelectricity, and now there is a revival of a bioelectrical view of biological systems.

Several studies show that bioelectrical signals are the key factor of cell–cell interaction in human body [58, 59]. Bioelectricity can underpin efficient growth and organization, morphogenesis, and regeneration in organisms [60, 61]. These findings have resulted in the realization that externally applied electrical fields can modulate multicellular processes [62, 63]. We argue in this review that bioelectricity can lead to a fundamental understanding of cellular activities, beyond its roles in the multicellular context. By cellular activities, we refer to high-level processes such as proliferation, dormancy, and differentiation that are underpinned by dynamic changes in gene expression, metabolic flux switching, and mechanical cell properties. Notably, these changes are linked to the integrated physio-chemical properties of the electrochemically active cellular environment interface. This

motivates a bioelectrical view of the cell, the development of which can lead to a predictive understanding of cellular activation, ultimately, for the physiological changes and therapeutic outcomes.

As above mentioned before, electrical stimulation can contribute to molecular and cellular activation under microcurrent (less than 1 mA) level. The physiological changes from the MCS rely on the cascading effects which are from molecular and cellular reactions to therapeutic phenomena in the whole body [64]. The diverse studies report that MCS can influence cellular signaling pathways [65], modulating intracellular processes [13], ion channel activity [4], and gene expression [66]. This impact on the cellular milieu is associated with the promotion of cellular regeneration and tissue repair [67, 68], and facilitating various physiological responses [69].

From the molecular point of view, MCS has the abilities to enhance the movement of charged or uncharged biomolecules across biological membranes through electrophoresis and electroosmosis. These combined processes are referred to as iontophoresis [70]. Iontophoresis is a widely used technique in transdermal drug delivery, particularly for enhancing the absorption of ionized drugs into the body with the assistance of electric current. This technique has been employed clinically for decades [71]. Recently, it has evolved and been studied as an iontophoresis technique combined with MCS utilizing nanomaterials such as nanoparticles for drug delivery [70].

Meanwhile, the electric activity of cells can regulate a variety of cell functions including growth, adhesion, differentiation, proliferation, activation of intracellular pathways, secretion of proteins, and gene expression [24]. Moreover, it can also regulate gene expression, through a variety of mechanisms such as the modulation of transcription factors, epigenetic modifications, and the regulation of mRNA stability. It has been additionally reported that MCS affects various intracellular signaling cascades including PI3K, cAMP, PTEN, ERK1/2, and calcium signaling [53, 64, 72].

Until this point, we understand that the mechanism of the phenomenon is related to the membrane potential. The membrane potential of a cell is the electrical potential difference between the inside and outside of the cell, which is maintained by the balance of ions across the cell membrane [73]. External electrical stimulation can cause changes in this balance of ions, which can result in a change in the membrane potential [74]. This change in membrane potential can then trigger the activation or inhibition of ion channels [75].

The cellular responses to MCS are still actively researched, and several hypotheses have been

proposed to explain these responses [7, 76]. In brief, several mechanisms collectively contribute to various cellular processes and pathophysiological functions. Several studies evaluated the physiological effects of MCS by focusing on the AMP-activated protein kinase (AMPK), which is closely related to voltage-gated calcium (Ca^{2+}) channels [77, 78]. The activation or inhibition of these ion channels can have various effects on the cell, such as changing its excitability or altering the release of neurotransmitters. In other words, MCS may modulate the opening and closing of these ion channels, which can lead to a variety of physiological effects [79]. To further extend our knowledge, we briefly addressed the AMPK and calcium (Ca^{2+}) channels below.

3.2 Mediation of enzyme to regulate inflammation and metabolism via MCS

Enzyme, representative AMPK, plays a central role in regulating cellular energy metabolism by monitoring the AMP/ATP ratio [80]. When cellular energy levels are low, AMPK is activated, leading to the activation of catabolic pathways that generate ATP and the inhibition of anabolic pathways that consume ATP. Moreover, AMPK regulates inflammation by modulating the activity of various signaling pathways for immune responses [81]. In brief, AMPK inhibits the activity of nuclear factor-kappa B (NF- κ B), and therefore plays a role as an inflammatory response. In addition to its role in regulating inflammation and metabolism, AMPK can mediate cell growth and differentiation, autophagy, and mitochondrial biogenesis [82]. Dysregulation of AMPK signaling has been implicated in various diseases, including metabolic disorders such as diabetes and obesity, neurodegenerative diseases, and cancer.

Overall, AMPK is a multifunctional enzyme that plays a central role in the regulation of cellular energy metabolism, inflammation, and various cellular processes. Its tight regulation and pleiotropic effects make it a promising target for therapeutic interventions in various diseases [83, 84].

There are several trials to elucidate the relation between MCS and AMPK pathway for therapeutic purposes [78, 85, 86]. Ran et al. observed the effect of electric field on CD9 expression and keratinocytes migration. From the results, they conclude that the electric field regulates CD9 expression and keratinocytes directional migration, in which AMPK pathway plays an important role in the wound healing [85]. Moreover, Bermeo et al. reported the role of AMPK in a maintenance and regulation of the voltage-gated calcium

channels. This study supports the correlation between AMPK with the regulation of Ca^{2+} channels and external stimulation concerning food intake and energy balance [78].

3.3 Inter-connection between influx of ion channel and representative enzyme

Living organisms, in common with human body, expend a significant proportion of their energy generating electricity [87]. Every cell maintains a voltage across its external membrane, and across the membranes of its organelles [88, 89].

Aggregates of cells also set up voltages across various tissue layers, cutaneous and corneal epithelium, vascular and intestinal walls, and the cortex and periosteum of long bones [88–94]. These voltages are of the order of millivolts (mV) in magnitude, and where there is a conducting pathway they cause the movement of ions within the tissue, constituting a bioelectric current, typically in the microcurrent range.

Among the ion channels, particularly, Ca^{2+} channels are membrane proteins that mediate the influx of Ca^{2+} ions into cells in response to various stimulation. The interconnection between Ca^{2+} channels and AMPK involves the regulation of cellular energy metabolism [95]. As mentioned above, AMPK is activated when cellular energy levels are low, leading to the activation of catabolic pathways that generate ATP. While the activation of AMPK occurs, Ca^{2+} channel also regulates cellular energy metabolism [96].

There are plenty of previous studies that Ca^{2+} channels can activate AMPK via a calcium-dependent mechanism [77]. The influx of Ca^{2+} ions through Ca^{2+} channels can activate Ca^{2+} /calmodulin-dependent protein kinase kinase (CaMKK), which in turn phosphorylates and activates AMPK. In addition, Ca^{2+} can stimulate AMPK indirectly by activating the calcium/calmodulin-dependent phosphatase calcineurin, which dephosphorylates and activates AMPK. According to other researches, it can be also reported that AMPK can regulate Ca^{2+} channels by phosphorylating and inhibiting L-type Ca^{2+} channels, which reduces Ca^{2+} influx into cells [97].

From here, we can estimate that metabolism related to ion can be conceptualized as a bioelectrical process with a redox process in which electrons are transferred from the electron donor to the electron acceptor. To facilitate this process, cells use a variety of electron sources and sinks, including redox-active compounds, metals, and their oxides. Moreover, the feedback mechanism is also accessed to maintain cellular energy homeostasis by preventing excessive Ca^{2+} influx and preserving cellular energy reserves [98] and it can be modulated by MCS. This opens up the possibility of using electrons in cellular metabolism via MCS.

4 Evaluation of MCS using in-vitro experiment and application for the therapeutic purposes

There are numerous studies regarding the physiological or therapeutic effects of MCS using in-vitro experiments. For instance, Jaatinen et al. [99] demonstrated that the mouse myoblast cell line undergoes dramatic changes in cell morphology, viability, cell structure, and cell adhesion under pulsed monophasic currents. Kumar et al. [100] showed that the cell proliferation and osteogenic differentiation of pre-osteoblast could favorably be regulated under dynamic electric field conditions. Besides, the neurite outgrowth of neural stem cells (NSCs) could be improved by the specific condition of electrical stimulation [101] and the differentiation of NSCs also be manipulated [102].

A common estimation from the above studies is that it is a cellular strategy to maintain an optimal growth rate to overcome external stimulation. Although the evaluations provide a plausible rationalization of cellular activities, it is limited to clarify the optimal level of growth. Therefore, an alternative simpler way was proposed that increasing activation rates would result in an increased ATP/ADP ratio and the aforementioned AMPK [103]. The relationship between the activation of AMPK and the ATP/ADP ratio is an aspect of cellular energy sensing and regulation. There are some scenarios to elucidate this relationship. An increased activation of AMPK is typically associated with a higher AMP/ATP ratio or an elevated ADP/ATP ratio, indicating a cellular state of energy stress. When cellular energy levels are high, the ATP/ADP ratio is elevated. In this scenario, AMPK is less active because it is less likely to be phosphorylated and activated. Conversely, when cellular energy levels are low, the ATP/ADP ratio decreases. This triggers a series of events, including an increase in the concentration of AMP, which leads to the phosphorylation and activation of AMPK.

Although we briefly mentioned the relationship between ATP/ADP ratio and AMPK, there are additional approaches to evaluate the effects of MCS to modulate physiological changes for therapeutic purposes. Here, we would introduce some experimental trials for the evaluation of MCS for therapeutic purposes including our previous researches.

4.1 Activation in hair cells (hair follicle dermal papilla) for enhancing hair growth

The control of human hair follicle dermal papilla cells (HFDPC) proliferation is crucial for fostering hair growth

and follicle development. Recent research indicates that applying low levels of electrical stimulation can serve as a catalyst for enhancing cell proliferation [104, 105]. Our findings reveal a significant increase in HFDPC proliferation and a decrease in wound area in groups exposed to 25 μ A and 50 μ A of MCS in wound healing assay [106]. Additionally, cell cycle analysis indicates an augmentation in the proportion of cells in the S and G2/M phases due to this stimulation (Fig. 1).

Within the hair follicle cycle, consisting of anagen, catagen, telogen, and exogen phases, catagen is recognized as the apoptosis-driven regression phase [107]. Apoptosis in this phase is governed by Bcl-2 and BAX proteins, where a reduced Bcl-2/BAX ratio triggers the transition from anagen to catagen in the hair cycle [108, 109]. Our study showed that in the MCS-applied group, there was an increase in Bcl-2 protein expression, while BAX protein expression remained unchanged. These results implied that MCS promotes cell proliferation by modulating cell cycle progression and inhibiting cell apoptosis (Fig. 2).

Furthermore, we explored the impact of MCS on the PI3K/AKT/mTOR/FoxO1 pathway and Wnt/ β -catenin signaling, recognized as crucial signal pathways associated with hair growth. These pathways contribute to various physiological and pathological processes, including cell proliferation, differentiation, and migration [110–115]. Notably, cell migration is known to be stimulated by the activation of the PI3K/AKT/mTOR/FoxO1 pathway, playing a pivotal role in hair follicle growth. Our findings demonstrate that MCS treatment results in the phosphorylation of GSK3 β at ser-9, indicating the inactivation of GSK3 β and subsequent stabilization of β -catenin (Fig. 3). This process is likely associated with the activation of the PI3K/AKT/mTOR/FoxO1 pathway and the induction of the Wnt/ β -catenin signaling pathway. These outcomes suggest that the activated PI3K/AKT/mTOR/FoxO1 pathway and Wnt/ β -catenin signaling pathway induced by MCS may promote the proliferation and migration of HFDPC, playing a key role in hair growth.

To assess the effects of MCS on hair growth in a telogenic C57BL/6 mice model, we conducted animal experiments comparing MCS with minoxidil treatment (MXD), a conventional drug-based approach. The examination of black pigmentation onset on the dorsal skin revealed an earlier initiation in both the MCS and MXD groups compared to the Control group. However, visual hair shaft emergence occurred on day 8 in the MCS group and on day 10 in the MXD group, indicating a more effective promotion of hair growth in the anagen phase with MCS. Histological analysis using H&E staining on Day 14 demonstrated a significant increase in the number of follicles in the MCS group (Fig. 4).

To understand how MCS influences genes associated with hair growth promotion, we evaluated mRNA expression

levels of various growth factors in the dorsal skin. MCS activated Wnt molecules, including Wnt 5a, Wnt 10a, and Wnt 10b, implicated in early morphogenetic stages [116]. Additionally, FGFs such as FGF2, FGF7, and FGF10, involved in increasing the number and size of hair follicles [117], were activated by MCS. Moreover, there was a significant upregulation of IGF-1 and its receptor, IGF-1R, suggesting a contribution to prolonging the anagen phase and facilitating hair shaft differentiation during the process of hair growth [117, 118] (Fig. 5).

4.2 Regulation in skin cells (macrophages) for repairing skin inflammation

Skin inflammation associated with acne is mediated by an inflammatory cascade triggered by bacterial infection, particularly *P. acnes*. *P. acnes*, a Gram-negative anaerobic bacterium, plays a crucial role in initiating and sustaining the inflammatory response in acne [119]. TLR-2, a Toll-like receptor primarily responsive to Gram-positive bacteria, can be activated by various molecular structures like peptidoglycans (PGNs), lipoproteins, lipoteichoic acid, and lipopolysaccharides, which are common components of bacterial cell walls [120]. Previous research has indicated that *P. acnes* activates TLR2 signaling, leading to the activation of the NF- κ B pathway. This pathway is responsible for the expression of downstream genes, including pro-inflammatory cytokines, chemokines, and prostaglandins. Studies have shown that *P. acnes*-induced reactive oxygen species activate the NF- κ B and mitogen-activated protein kinase (MAPK) pathways, subsequently inducing the expression of inducible NO synthase (iNOS)/NO and cyclooxygenase-2/prostaglandin E2 (COX-2/PGE2) in macrophages [121]. Consequently, the inhibition of TLR2/NF- κ B signaling activation has been identified as a primary therapeutic target for anti-inflammatory strategies in *P. acnes*-induced skin inflammation [122–124].

We evaluated the anti-inflammatory effects of MCS at intensities of 50, 100, and 200 μ A based on nitric oxide (NO) production in PGN-treated RAW 264.7 macrophages. The 50 μ A intensity exhibited the lowest NO production rate, leading us to select 50 μ A as the micro-current stimulation intensity for subsequent experiments. We examined the changes in the protein expression levels of TLR2 and related proteins (MyD88, TRAF6, and p-TAK1) implicated in activating the canonical NF- κ B pathway. Upon micro-current stimulation, the expression levels of these proteins in the cells treated with both PGN and MCS (PGN/MC) were significantly reduced, showing similar levels to those of control (CON) group that was not treated with PGN. These suggest that micro-current stimulation may downregulate subsequent signaling, particularly canonical NF- κ B pathway activation (Fig. 6).

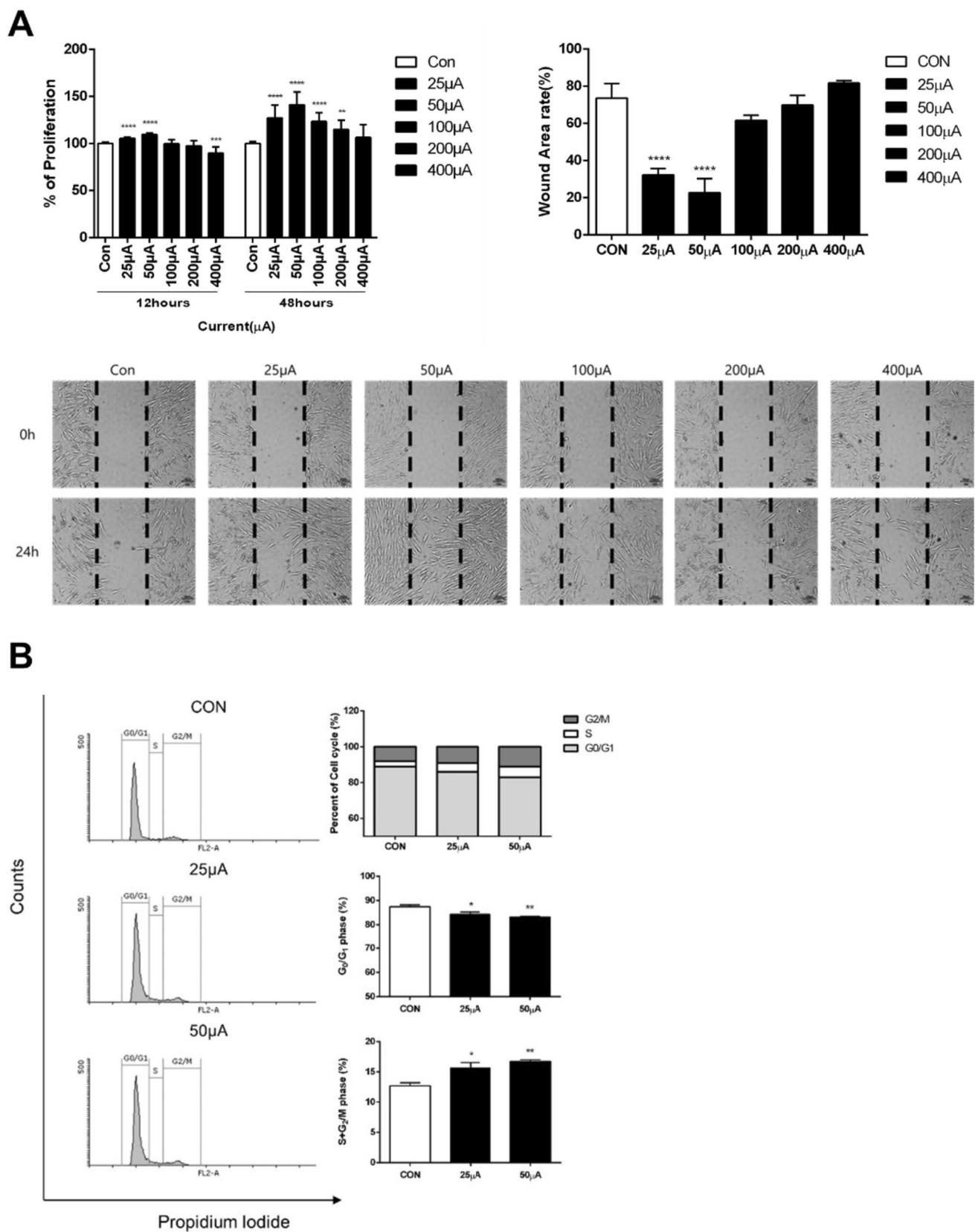


Fig. 1 **A** Effects of MCS on the proliferation of HFDPC cells and wound healing assay results. **B** Change in cell cycle distribution in HFDPC following the treatment of MCS. The population of cells in G₀/G₁, S, and G₂/M phases in microcurrent stimulation-treated HFDPC [106]

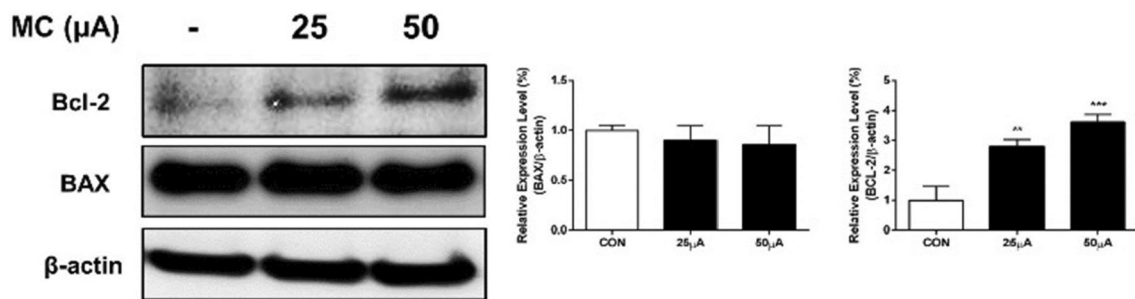


Fig. 2 Immunoblot analysis of cell apoptosis-related proteins on HFDPC following the application of MCS [106]

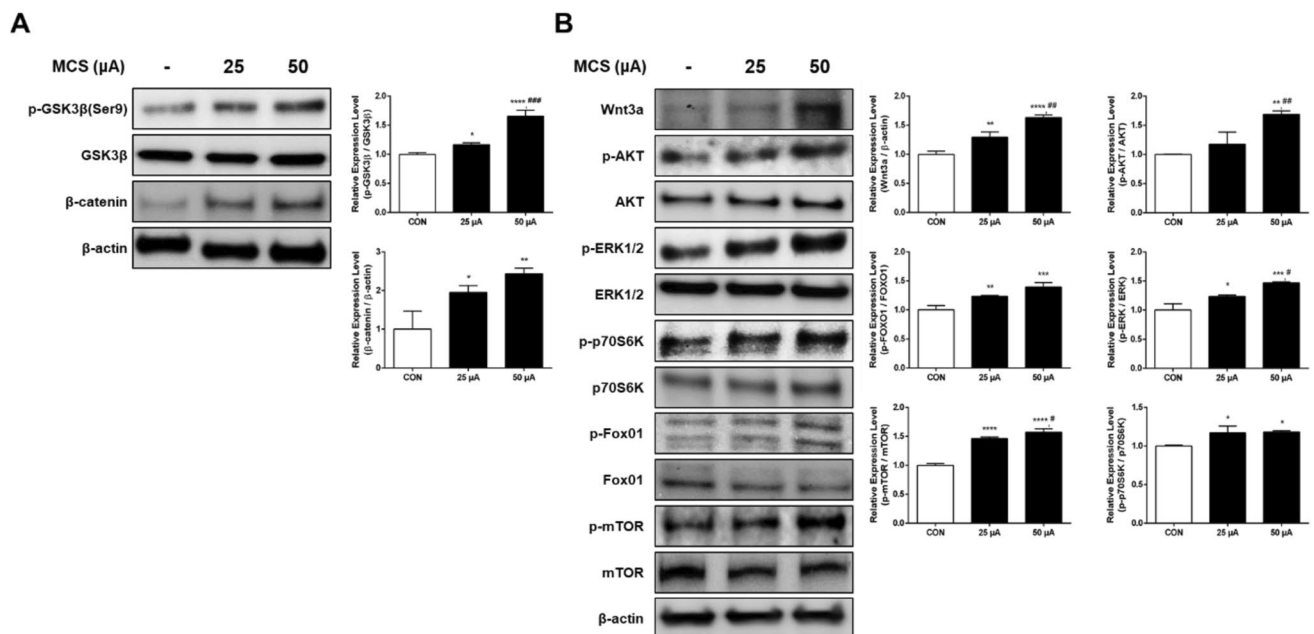


Fig. 3 **A** Immunoblot analysis of the protein expressions regarding GSK3β/β-catenin signaling pathway in HFDPC cells following treatment with MCS. **B** Immunoblot analysis of the protein expressions

regarding PI3K/AKT/mTOR/FoxO1 signaling pathway in HFDPC cells following treatment with MCS [106]

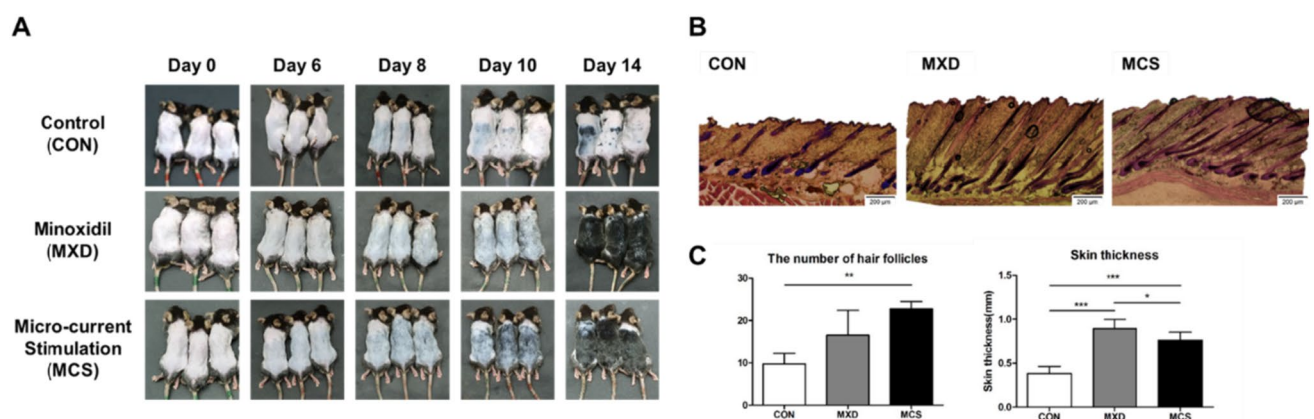


Fig. 4 **A** Photographs of shaved dorsal skin were taken at Day 0, 6, 8, 10, and 14 in control group (CON), Minoxidil-treated group (MXD) used as a positive control, and micro-current stimulation with the

intensity of 50μA group (MCS). **B** Longitudinal sections of the dorsal skins for each group by H&E staining. **C** The number of hair follicles and skin thickness (Full thickness) in the section for each group [106]

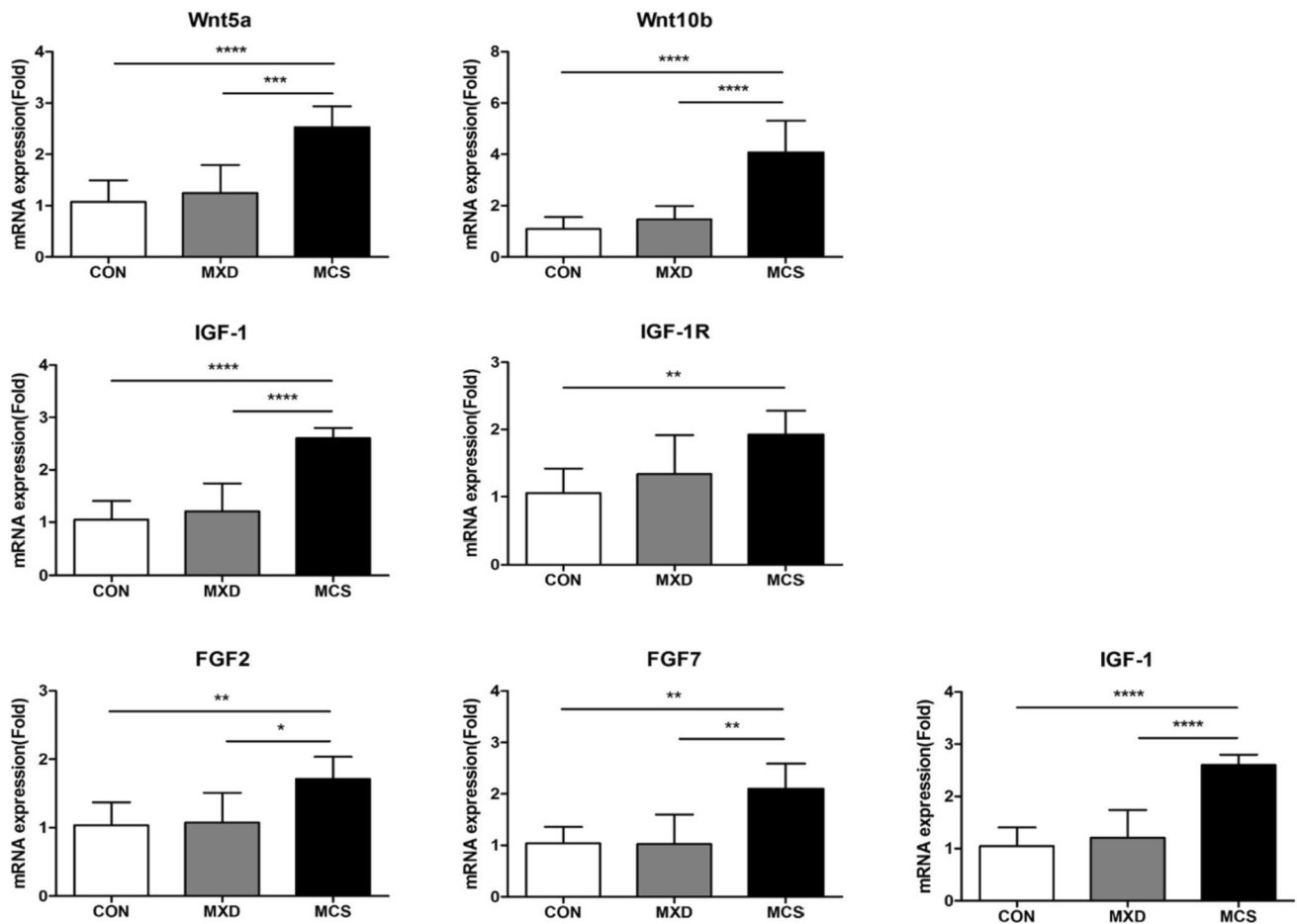


Fig. 5 mRNA expression levels of growth factors contributing to telogen-anagen transition and hair growth promotion in telogenic mice [106]

To further understand the influence of micro-current stimulation on this signaling pathway, our study investigated the expression of NF- κ B-related proteins through western blotting and the localization of NF- κ B p65 through immunofluorescence staining (Fig. 7). The expression of p-NF- κ B significantly increased, while I κ B α expression decreased in the PGN group. Conversely, the application of MC not only decreased p-NF- κ B protein expression but also increased I κ B α expression. Consistent with these findings, immunofluorescence staining results indicated that NF- κ B p65-positive staining, primarily located in the cytoplasm with PGN, shifted to the nucleus. Notably, MCS reduced PGN-induced translocation of NF- κ B p65, suggesting that MCS can modulate the inflammatory signaling cascade by regulating NF- κ B activation. In the interplay between TLR2 and *P. acnes*, NF- κ B serves as a crucial factor influencing inflammatory responses in acne, releasing pro-inflammatory cytokines like COX-2, iNOS, IL-1 β , and TNF- α [126]. These cytokines play a significant role in acne-associated follicular hyperkeratinization and inflammatory responses. Our data revealed that PGN increased the protein-level expression of

these cytokines in Raw 264.7 cells, and notably, the application of MCS significantly suppressed the expression of these pro-inflammatory cytokines.

To assess the therapeutic effects MCS in vivo, we used a mouse model injected with *P. acnes*, a well-established animal model for acne [127]. H&E staining results revealed significantly reduced swelling, infiltrated inflammatory cell count, and granulomatous response in MCS-applied lesions compared to lesions injected solely with *P. acnes*. Inflammation-associated cytokines like TNF- α , IL-1 β , and COX-2 play roles in follicular hyperkeratinization and acne lesion development [128]. TNF- α , expressed in early inflammatory stages, induces responses like vasodilation, edema, and fever [129, 130]. IL-1 β contributes to inflammatory papules, pustules, and nodules in acne, with its production mediated by *P. acnes*-induced NLRP3-inflammasome activation [131]. COX-2, an inflammatory mediator, is pivotal in prostaglandin and lipid mediator production, influencing inflammatory responses. Increased COX-2 production leads to sebaceous gland hyperplasia and elevated

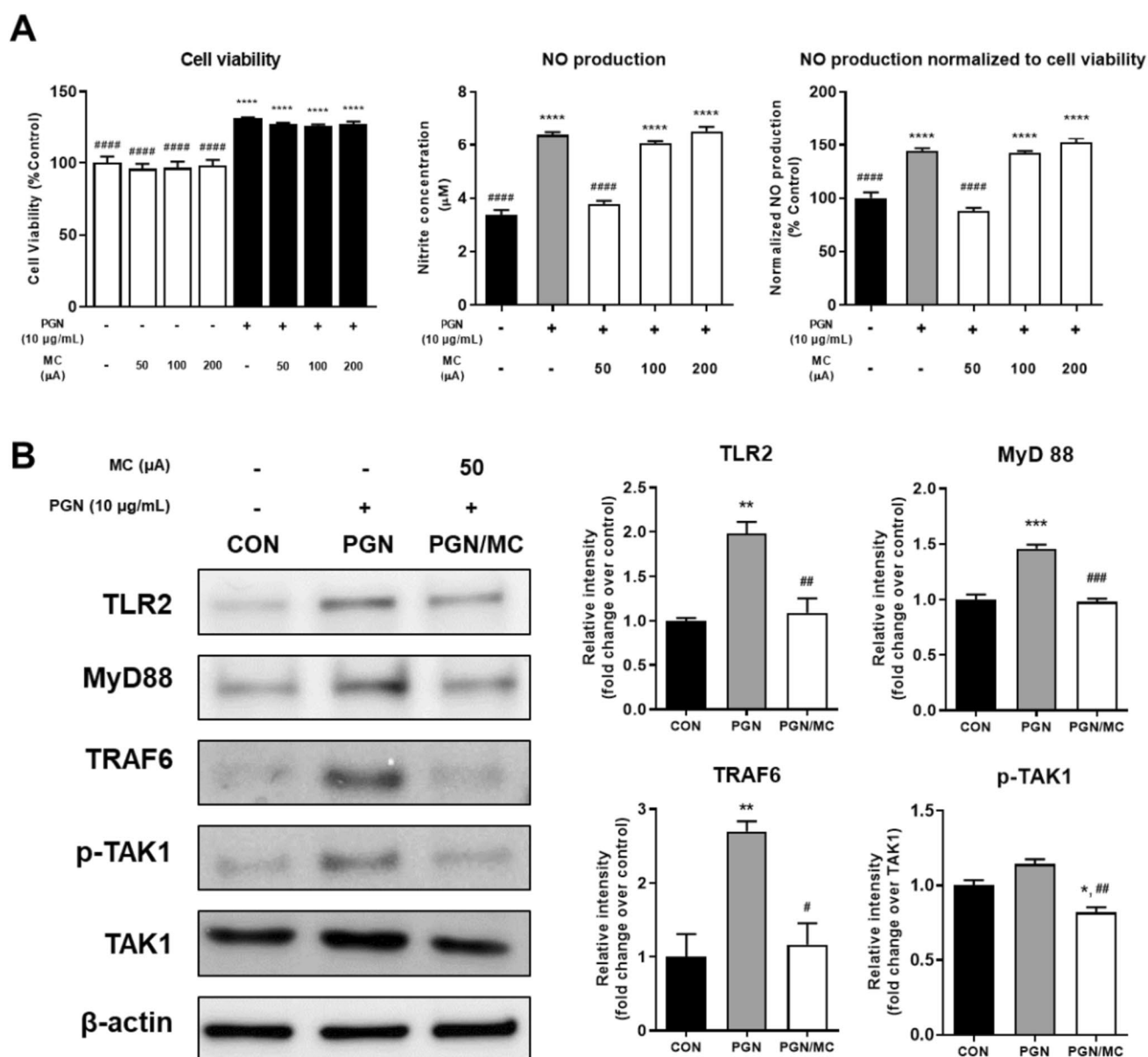


Fig. 6 **A** Cell viability and NO production according to the presence or absence of PGN treatment or various levels of MCS in Raw 264.7 cells. **B** Immunoblot analysis of TLR2-related proteins on Raw 264.7 cells following the application of MCS [125]

sebum production, crucial in sebocyte biology related to acne. MCS significantly inhibited TNF- α , IL-1 β , and COX-2 expression levels, consistent with in vitro findings. Particularly, there was a significantly lower IL-1 β level in MCS-applied animals compared to those in PGN group (Fig. 8). TLR-2, implicated in the secretion of these cytokines and mediators, was also significantly inhibited by MCS. These results can suggest a possible mechanism for MCS in PGN- or *P. acnes*-mediated inflammation (Fig. 9).

4.3 Application in fat cells (adipocytes) for inhibiting adipogenesis

The process of adipogenesis involves multiple steps, encompassing the proliferation and differentiation of precursor cells into mature adipocytes [132]. Adipose tissue expansion manifests through two mechanisms: hyperplasia, which involves an increase in the number of adipocytes, and hypertrophy, which refers to the enlargement of adipocytes [133]. Therefore, a potential therapeutic strategy for obesity

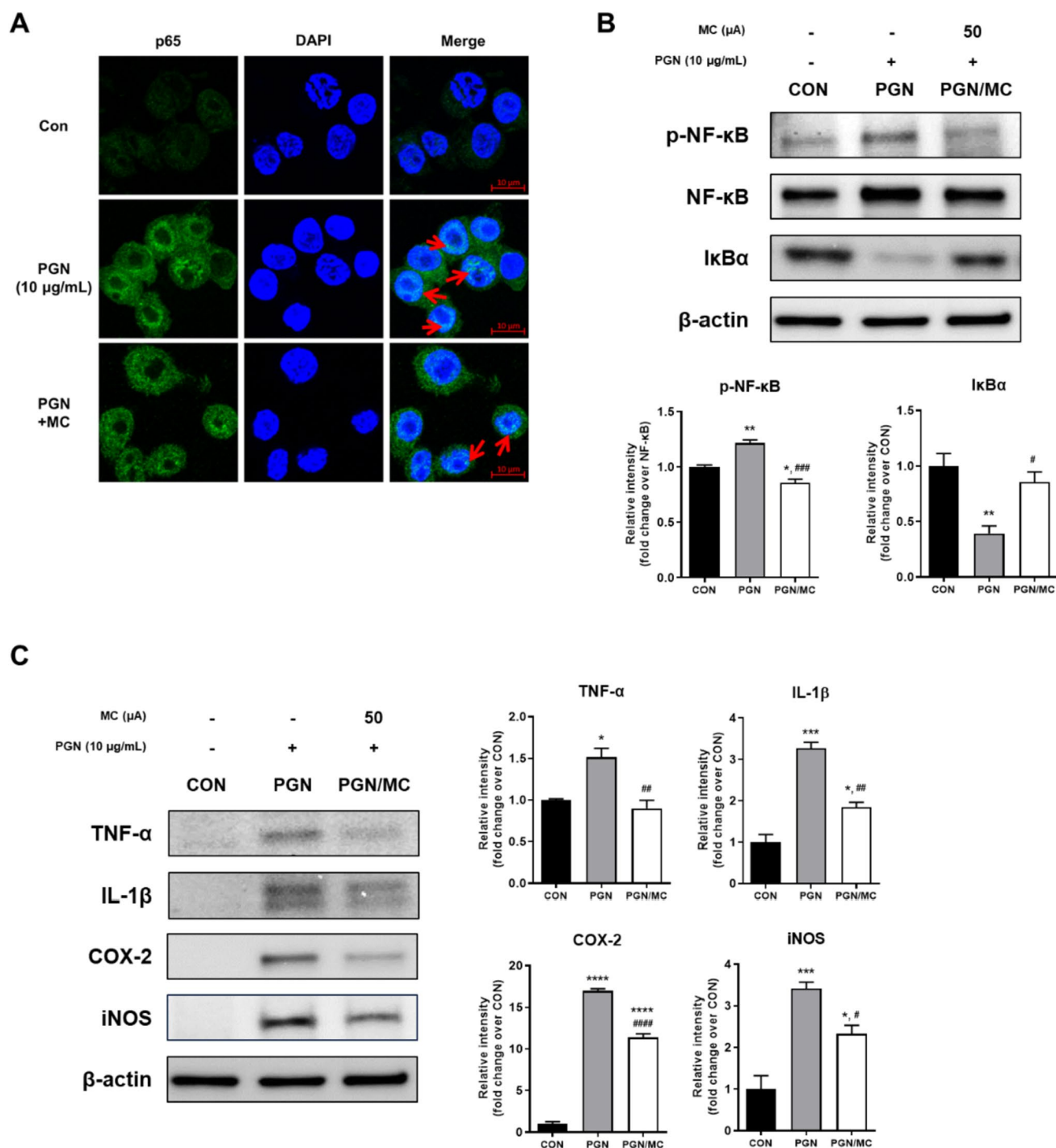


Fig. 7 **A** Confocal microscopy for observation of the translocation of NF-κB (p65) to the nucleus. **B** Immunoblot analysis of NF-κB-related proteins on Raw 264.7 cells following the application of MCS. **C**

Immunoblot analysis of pro-inflammatory cytokines or mediators on Raw 264.7 cells following the application of MCS [125]

could be to decrease the size or number of adipocytes by regulating adipogenesis.

We applied MCS for one hour just before the preadipocytes' differentiation phase to inhibit differentiation into adipocytes, which corresponds to hyperplasia. Following

this, we induced preadipocyte differentiation and, six days later, measured intracellular lipid accumulation using Oil Red O staining to examine the impact on lipid content. Among the various MCS intensities (25, 50, 100, 200, and

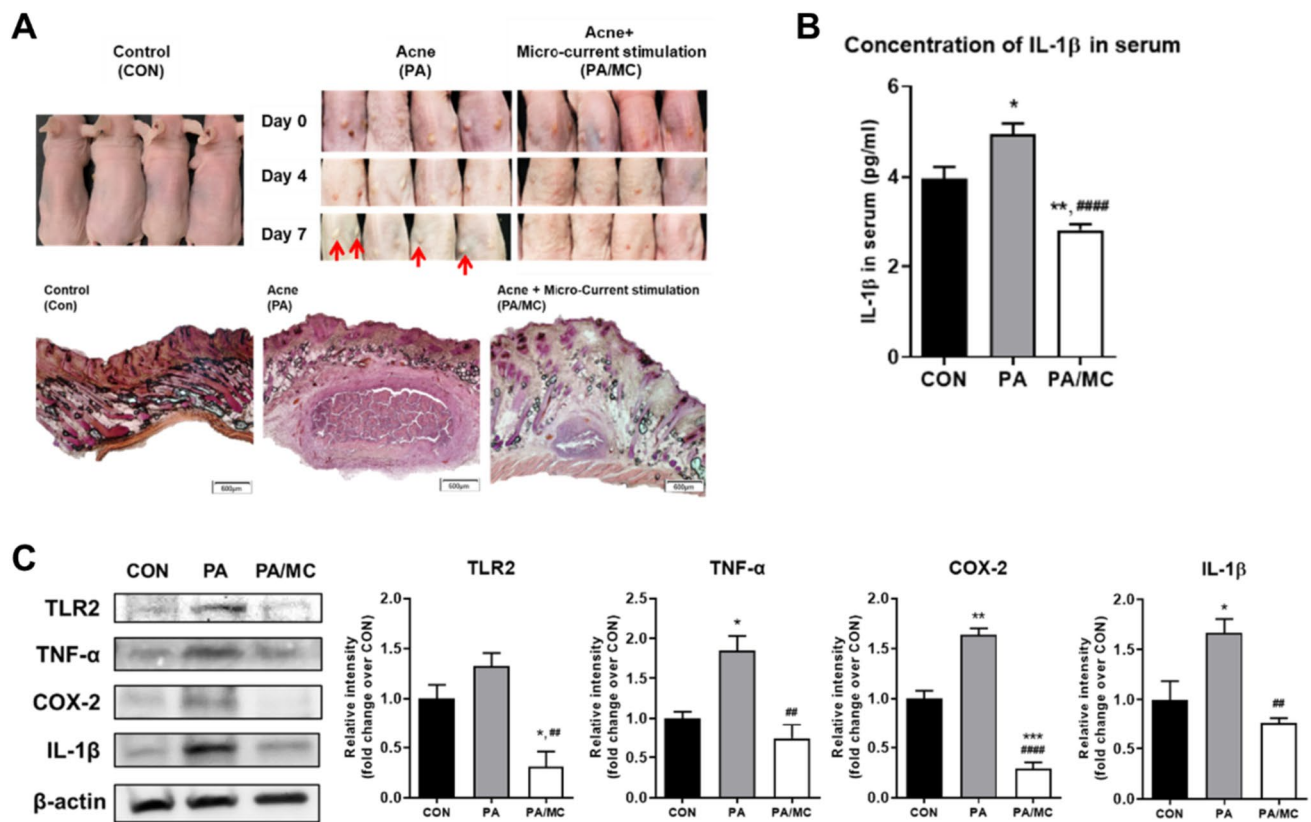
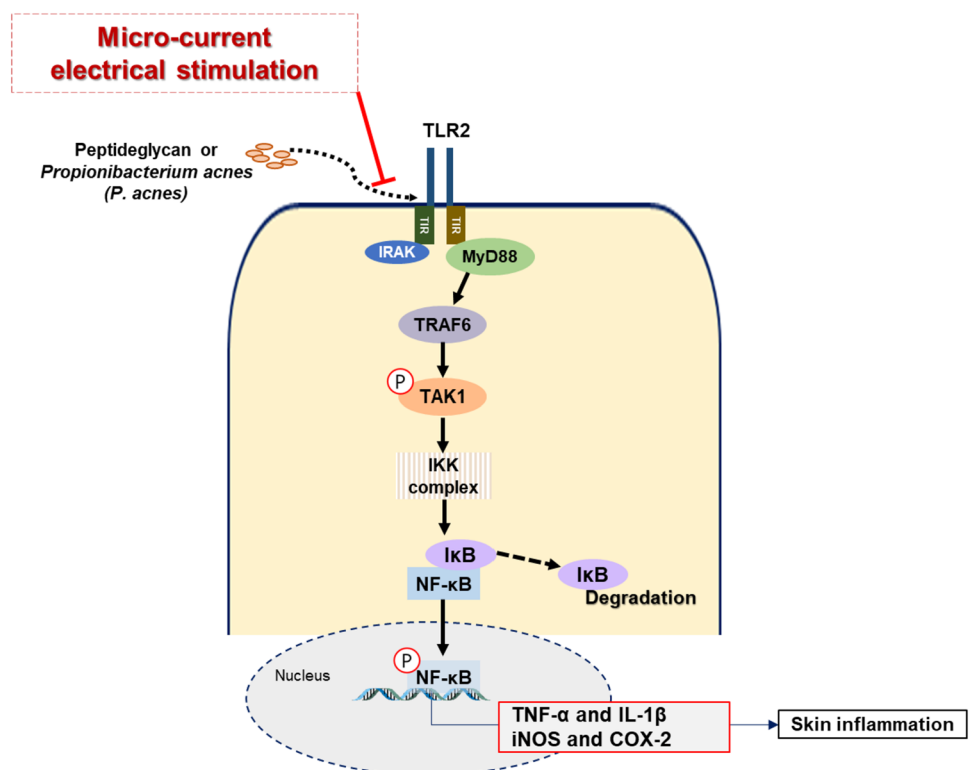


Fig. 8 **A** Photographs of dorsal skin and longitudinal sections of the individual lesions for each group by H&E staining. **B** Concentration of IL-1 β in serum. **C** Immunoblot analysis of TLR2 and pro-inflammatory cytokines or mediators on the tissue of acne lesions [125]

Fig. 9 Schematic diagram for the anti-inflammatory effects of MCS on PGN or *P. acnes*-induced inflammatory responses via downregulating TLR2/NF- κ B signaling pathways [125]



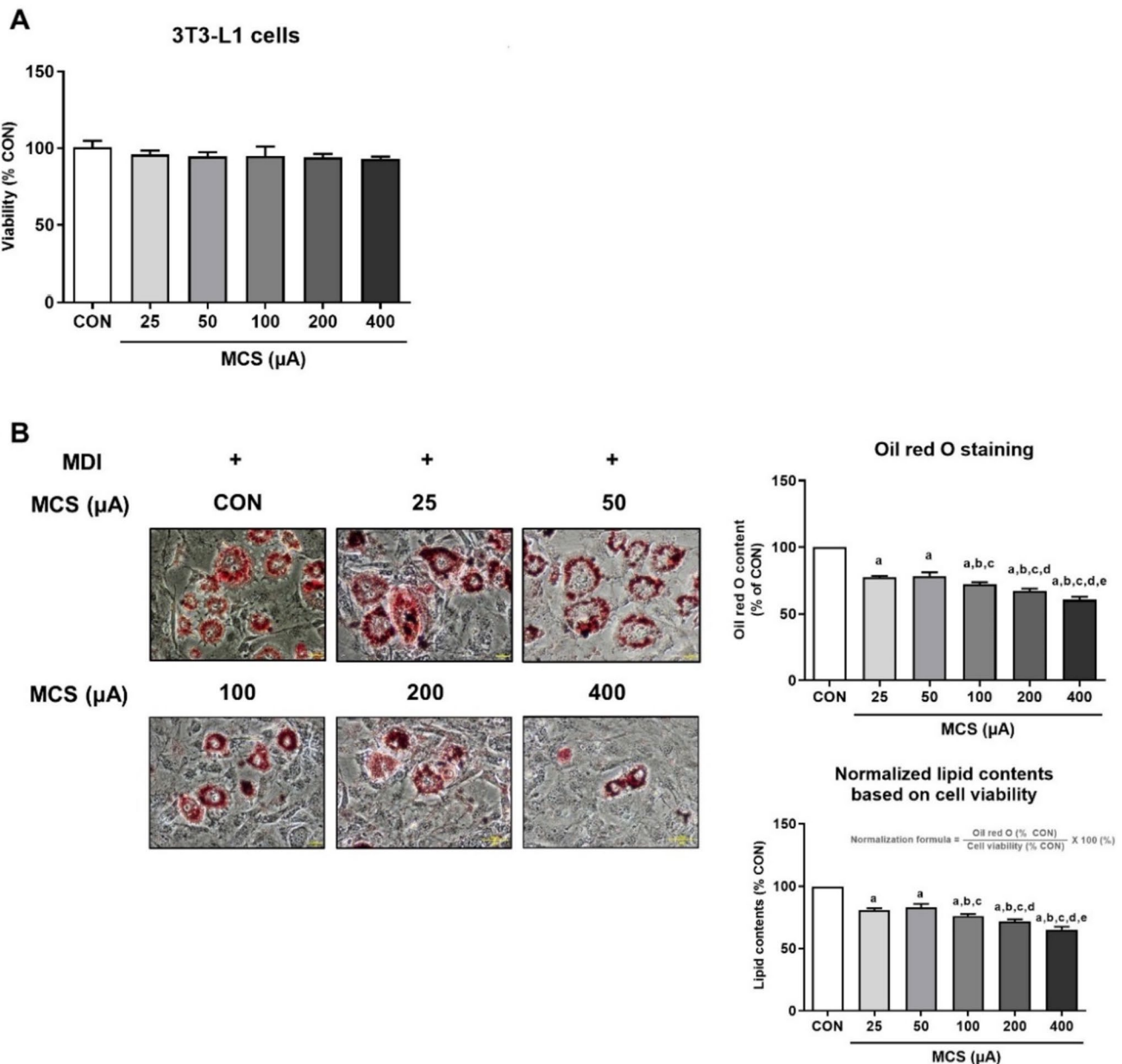


Fig. 10 **A** The cytotoxicity of 3T3–L1 cells was measured by the application of MCS (25, 50, 100, 200, and 400 μA). **B** Microscopic images of Oil Red O stained 3T3–L1 cells and quantification of lipid droplet (magnification: 200X) [10]

400 μA), 200 μA and 400 μA demonstrated significantly lower levels of lipid accumulation (Fig. 10).

The phosphorylation of insulin receptor subunits and subsequent lipid accumulation are mediated through the insulin signaling pathway in 3T3–L1 cells [134]. Insulin receptors and insulin-like growth factor receptors undergo phosphorylation upon insulin treatment [135]. To verify the effect of MCS on inhibiting lipid accumulation, we investigated whether MCS could modulate insulin signaling. The application of MCS was found to inhibit the phosphorylation of insulin signaling pathway proteins such as IR, IGF, ERK,

and Akt. Additionally, it was confirmed that MCS resulted in a reduction in the expression levels of C/EBP-α and PPAR-γ, recognized as transcription factors involved in adipogenesis (Fig. 11).

Recently, several studies have reported that the WNT/β-catenin pathway can effectively inhibit adipocyte formation by regulating PPARγ and C/EBPα, which are key transcription factors in adipocyte formation [136]. MCS inhibited adipocyte differentiation by increasing the activation of the Wnt/β-catenin pathway and decreased the expression of C/EBP-α (Fig. 12). When Wnts, an

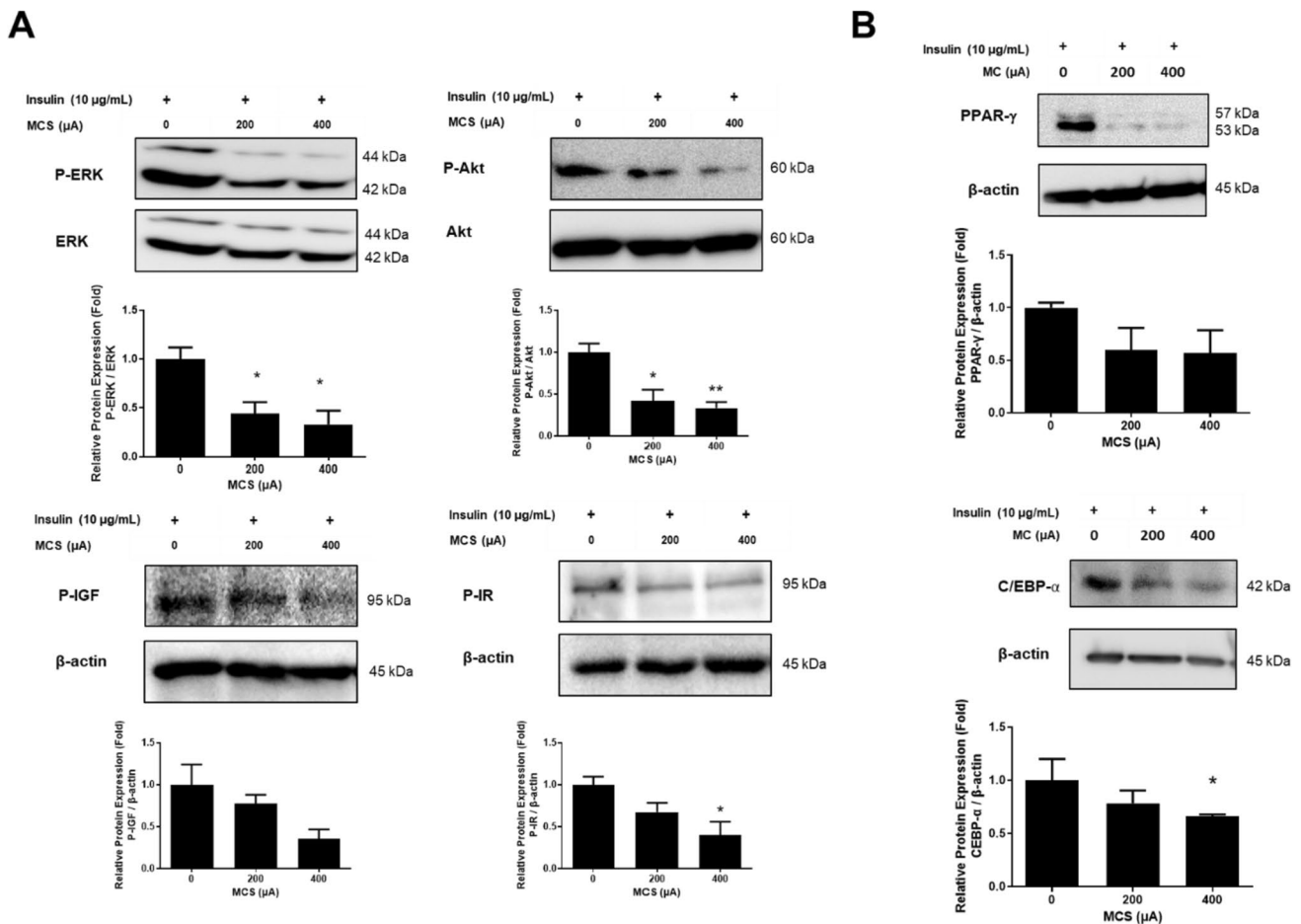


Fig. 11 **A** Immunoblot analysis of insulin signaling pathway proteins such as IR, IGF, ERK, and Akt in 3T3-L1 cells. **B** The protein expression of PPAR-γ and C/EBP-α in 3T3-L1 cells [10]

extracellular glycoprotein, binds to the frizzled (FZ) and low-density lipoprotein receptor-related protein (LRP) receptors, it induces the activity of disheveled (DVL), glycogen synthase kinase (GSK) 3β, and adenomatous polyposis coli (APC), and AXIN complex [137, 138]. Consequently, the degradation of β-catenin is suppressed and its movement to the nucleus increases, and it can promote the expression of various target genes such as cyclin D1 (CCND1), which inhibits the expression of PPARγ and C/EBPα, through binding to T cell factor/Lymphoid enhancer factor (TCF/LEF) [139, 140].

Studies using animal models mainly used leptin-deficient ob/ob obese mouse models, and similar to the in-vitro results mentioned above, abdominal adipose tissue volume was significantly reduced at 200 and 400 µA. Visual observation of the epididymal fat pad, where white adipose tissue is mainly distributed, showed the same trend, and it was confirmed that the protein expression level of C/EBP-α in adipose tissue was also reduced (Fig. 13).

In addition, the size and weight of liver tissue were also significantly reduced, and blood indicators showed a decrease in serum triglycerides (Fig. 14). Based on these changes, these studies predicted that MCS would also be related to metabolic functions of the liver.

4.4 Application in hepatic cells (hepatocyte) for non-alcoholic fatty liver diseases

Excessive lipid accumulation in liver tissue interferes with the normal physiological functions of hepatocytes, resulting in abnormalities in lipid and carbohydrate metabolism, hepatocytes inflammation, and insulin resistance [143, 144]. If the disease worsens, it can lead to diabetes, chronic liver fibrosis, and liver cancer, so it is important to prevent the worsening or quickly improve the initial stage of liver steatosis [145]. According to previous studies, strategies to improve hepatic steatosis include suppressing lipid accumulation in the liver, decomposing fat, and promoting fatty acid beta-oxidation metabolism.

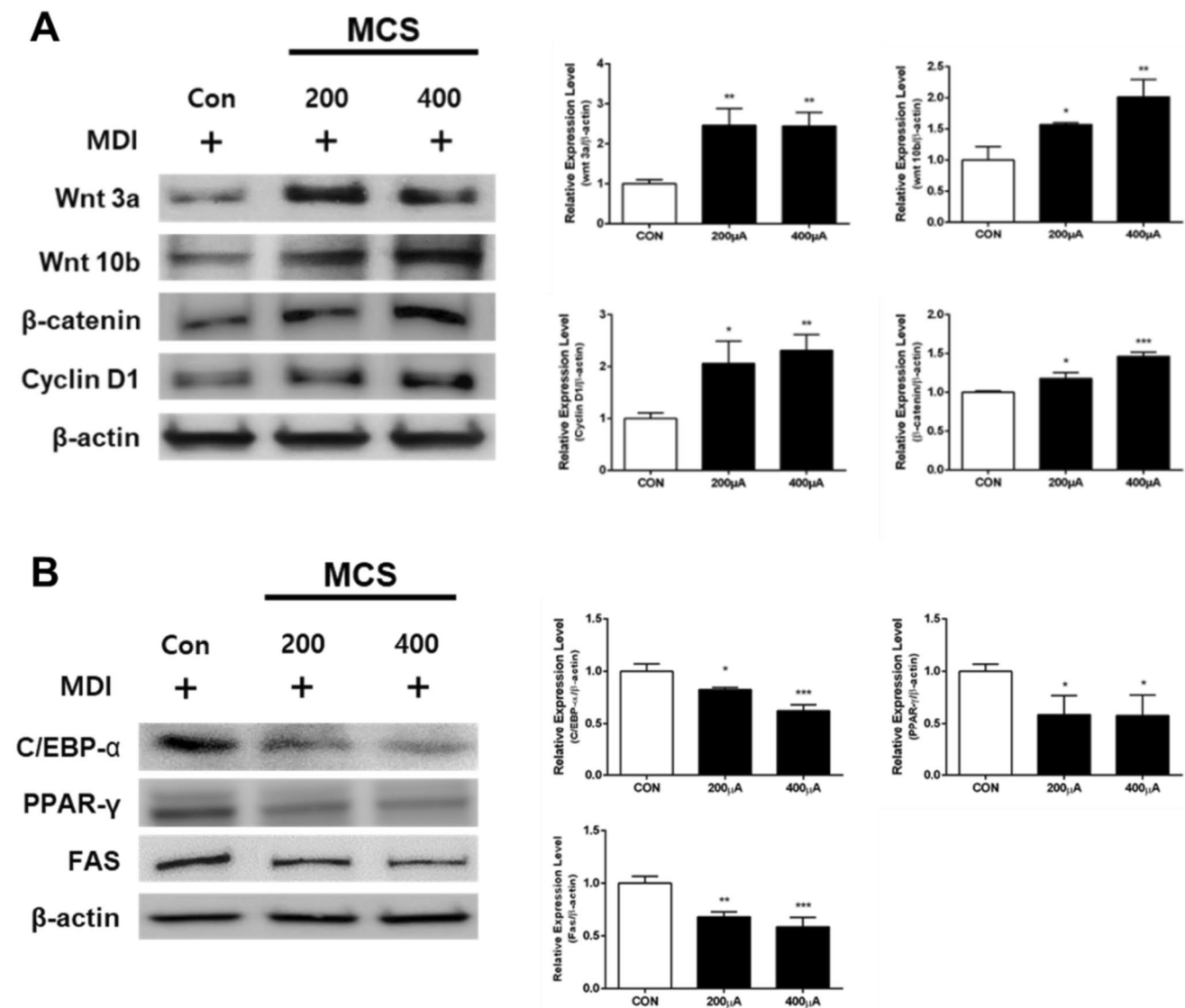


Fig. 12 **A** Immunoblot analysis of Wnt Signaling Pathway in 3T3-L1 cells. **B** The protein expression of adipogenic transcription factors such as C/EBP-α, PPAR-γ, and FAS in 3T3-L1 cells [141, 142]

To select an MCS intensity that showed the potential to improve non-alcoholic fatty liver diseases (NAFLD), the amount of lipid contents accumulated in hepatocytes was compared according to whether oleic acid (OA) treatment and MCS were applied through Oil red O staining (Fig. 15). The amount of lipid accumulation increased in the OA group where lipid accumulation was induced by OA. The MCS intensities used in the experiment were 50, 100, 200, and 400 μ A, and the lipid content decreased depending on the current intensities. Based on this, 200 μ A and 400 μ A, which showed the most effect, were used to observe changes in lipid metabolism.

Various studies have used activation of the Sirt1/AMPK signaling pathway as a method to improve non-alcoholic fatty liver disease and hepatic steatosis, which is known

to inhibit lipid accumulation and promote lipolysis and fatty acid oxidation [147, 148]. AMPK is an important enzyme that senses and regulates cellular and systemic energy balance, and Sirt1 is a nicotinamide adenine dinucleotide (NAD)-dependent deacetylase that is known to regulate lipid metabolism by activating AMPK expression [149, 150]. In particular, AMPK α activation can reduce liver fat accumulation by inhibiting lipid biosynthesis and accelerating lipolysis [151]. When AMPK α is activated, it induces phosphorylation of ACC and reduces ACC activity, thereby inhibiting lipid biosynthesis [151, 152]. The results of this study showed that 400 μ A of MCS tended to activate the Sirt1/AMPK signaling pathway, and based on the results of this study, it was predicted that fatty acid

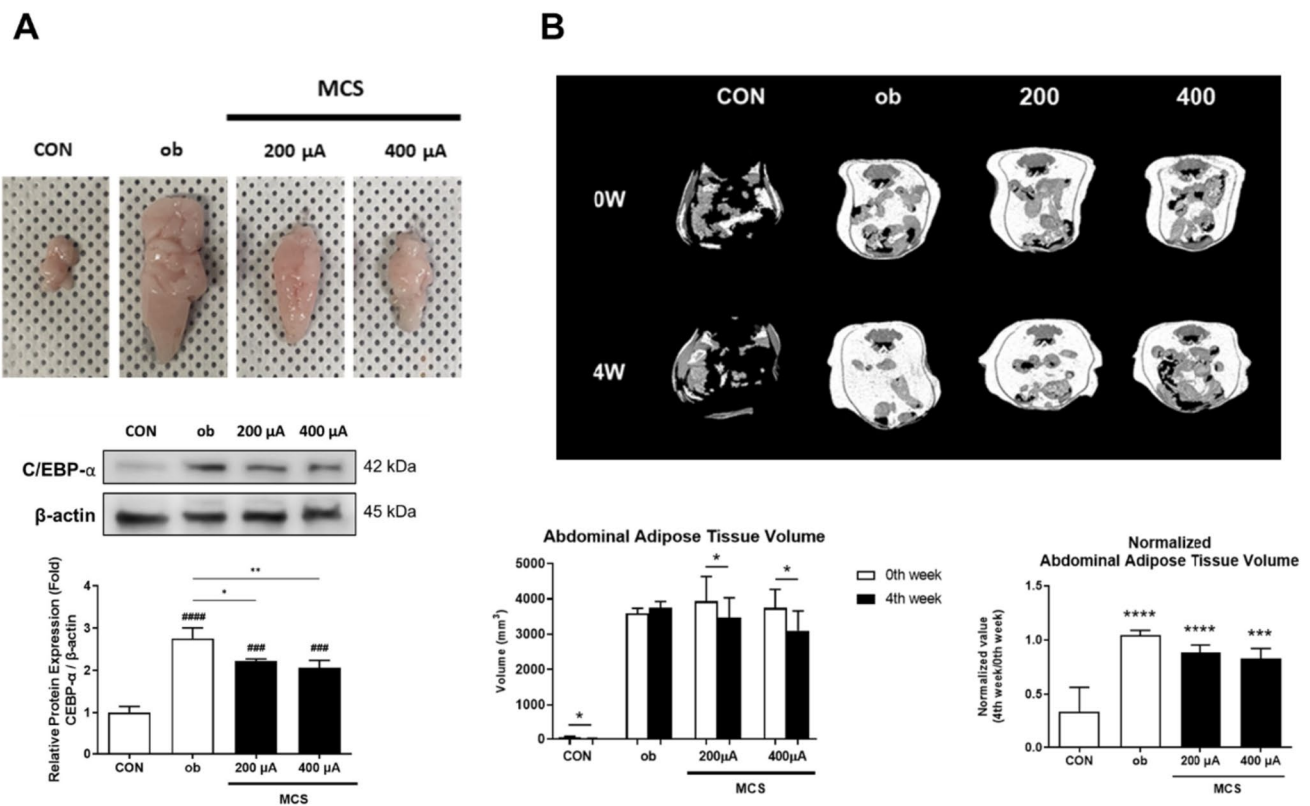


Fig. 13 **A** The appearance of the epididymal fat pad and the protein expression of C/EBP- α in epididymal fat tissue. **B** Representative 3D images of abdominal adipose tissue acquired from Micro-CT and changes in the abdominal adipose tissue volume [10]

beta-oxidation would be promoted by activating the Sirt1/AMPK signaling pathway (Fig. 16).

When the accumulated neutral fat in the liver cells is broken down, free fatty acids are finally released. If the released free fatty acids are not broken down immediately, it causes an inflammatory response in hepatocytes, macrophages, and vascular endothelial cells, and activated macrophages are inflammatory. It is known to induce insulin resistance in hepatocytes by releasing mediators [153, 154]. These free fatty acids can be immediately decomposed through the fatty acid beta-oxidation process [155]. Among the factors involved in this process, PPAR α is a transcription factor that promotes beta-oxidation and directly controls genes related to peroxisome and mitochondrial beta-oxidation pathways, fatty acid absorption, and neutral fat catabolism [156–158]. PGC1 α acts as a coactivator of PPAR α , and the PGC1 α -PPAR α complex is known to play a key role in beta oxidation [159]. The results of this study showed that PGC1 α was activated in both OA200 and OA400, whereas PPAR α was activated only in OA400 (Fig. 17).

In addition, to alleviate NAFLD, a condition in which lipids have already accumulated in liver tissue, methods to promote lipolysis in liver tissue must be considered. Hepatocytes are known to produce glycerol and free

fatty acids through the citric acid cycle for triglyceride decomposition and energy production [160]. The process of neutral fat decomposition goes through the hydrolysis process of neutral fat $\rightarrow$ diglyceride $\rightarrow$ monoglyceride [154, 161]. As a result of measuring the expression level of ATGL, which is involved in hydrolyzing neutral fat into diglyceride, both 200 and 400 μ A showed increased activity compared to CON (Fig. 18). However, the results of comparison with OA showed a significantly higher expression level only at 400 μ A, so the stimulation intensity for sufficient effect in NAFLD state appears to be 400 μ A. On the other hand, it is known that lipolysis is inhibited when HSL (Ser565), whose expression is regulated by AMPK, is phosphorylated [162, 163]. When related to the activity of AMPK as seen above, it can be seen that the expression level of p-HSL (Ser565) is reduced in OA400, which showed relatively active p-AMPK. This suggests that lipolysis can be promoted at 400 μ A. Ultimately, MGL, which is involved in the decomposition of monoglyceride into glycerol and free fatty acid, showed increased activity compared to CON at both 200 and 400 μ A, similar to the ATGL results, and showed a significant difference only at 400 μ A in comparison with OA. Summarizing the results of this study on lipolytic enzymes, it can be seen

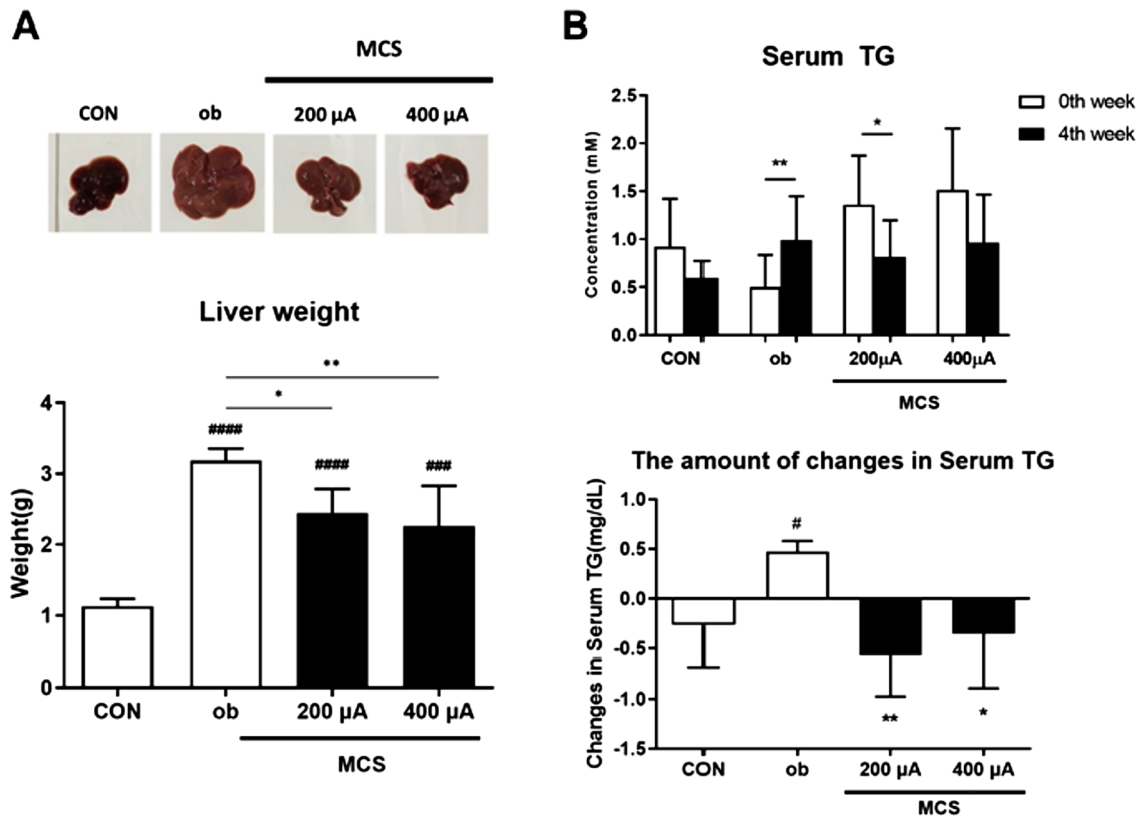


Fig. 14 **A** The appearance and weight of the liver. **B** Changes in serum triglycerides (TG) levels at before and after the experiment [10]

that compared to OA, it is activated only at an MCS of 400 μ A, so the effect of 200 μ A on lipolysis is low.

Previous studies have reported that AMPK phosphorylation can inhibit the expression of adipogenesis-related transcription factors such as SREBP-1c and PPAR, and suppressing the expression of adipogenesis-related factors may be one way to prevent the worsening of NAFLD. The expression levels of SREBP-1c, PPAR γ , and FAS were examined as adipogenesis-related factors (Fig. 19). SREBP-1c involves the synthesis of lipids and cholesterol and plays a role in activating FAS gene expression by binding to the FAS promoter [164]. Both FAS and SREBP-1c showed significantly higher expression levels in OA400. It was reported that PPAR γ , a major transcription factor involved in lipid synthesis and related gene activity, can reduce the expression of FAS, which is involved in adipogenesis [165]. As a result of comparing the expression level of PPAR γ in this study, a significant difference was found only in OA400 compared to OA, which also showed a similar trend to the FAS results. As a result, it was predicted that the electrical stimulation intensity of 400 μ A had the effect of suppressing lipid accumulation in hepatocytes by activating the expression of SREBP-1c, PPAR γ , and FAS, transcription factors related to fat synthesis.

Taken together, it was estimated that 400 μ A current stimulation can promote fatty acid beta-oxidation by activating the Sirt1/AMPK signaling pathway, and will be effective in promoting lipolysis and suppressing lipogenesis (Fig. 20).

5 Future directions of microcurrent stimulation beyond the tissue levels

In the above chapter, we addressed the therapeutic effects of MCS at molecular and cellular levels. Moving to the tissue and organ level, MCS is commonly applied in rehabilitation settings to enhance tissue function and physiological advantage. Its effects extend to connective tissues, where it may influence collagen synthesis and extracellular matrix production, contributing to tissue integrity [166, 167]. Furthermore, MCSs are suggested to enhance microcirculation, facilitating improved blood flow to the stimulated area and supporting nutrient delivery and waste removal [168, 169].

At the systemic level, MCS demonstrates utility in pain management, modulating pain perception through mechanisms like endorphin release and pain signal gating [170, 171]. Additionally, it is thought to exhibit anti-inflammatory effects [172, 173], impacting systemic

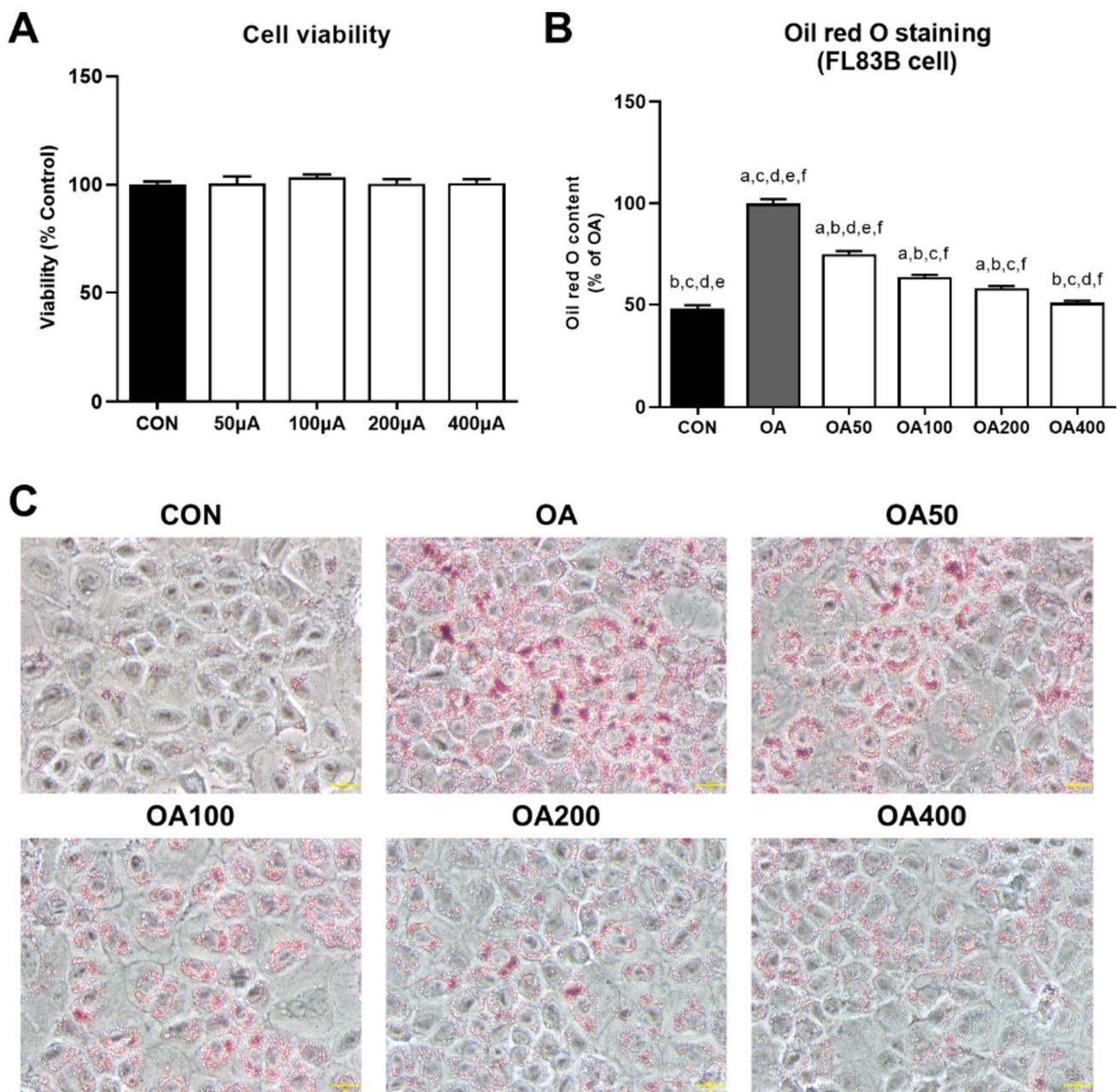


Fig. 15 **A** Cell viability of the MCS on FL83B cells. **B** Quantification of Oil-red O staining for each group. **C** Lipid droplets observed by microscope in FL83B cells stained Oil-red O [146]

inflammation levels and potentially offering benefits for conditions associated with chronic inflammation. The neurological effects of MCS may extend beyond the local area, influencing central nervous system activity and neuroplasticity.

Considering whole-body responses, MCS may influence overall energy balance by modulating cellular metabolism and energy production. Its potential impact on the stress response, including cortisol levels and stress-related pathways, suggests a broader systemic effect [174].

While ongoing research explores the interplay between microcurrent stimulation and immune function, some studies suggest that it may indeed modulate immune responses at the systemic level, potentially through the regulation of cytokines.

Additionally, several studies have shown that MCS can be beneficial for various skin-related symptoms, including wound healing, psoriasis, skin barrier formation, and facial care [175–178]. Additionally, it is reported that MCS has the ability to prevent the accumulation of lipids in cells by

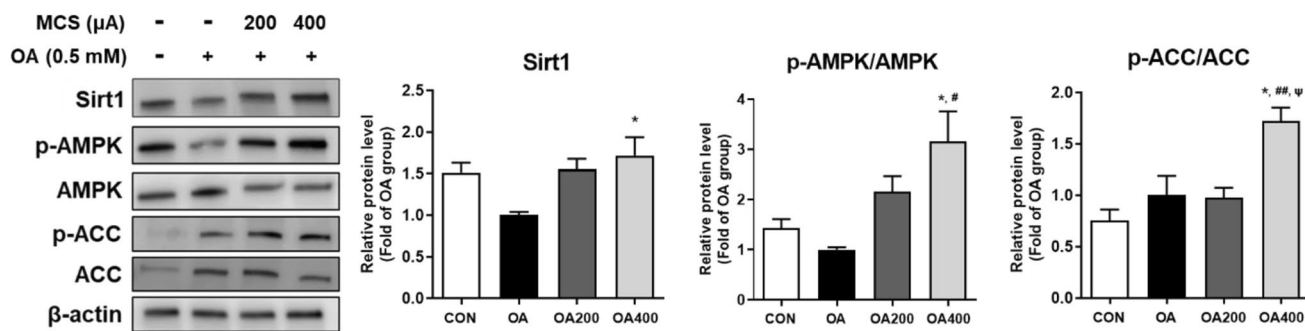


Fig. 16 Immunoblot analysis of the protein expression regarding Sirt1/AMPK pathway in FL83B cells [146]

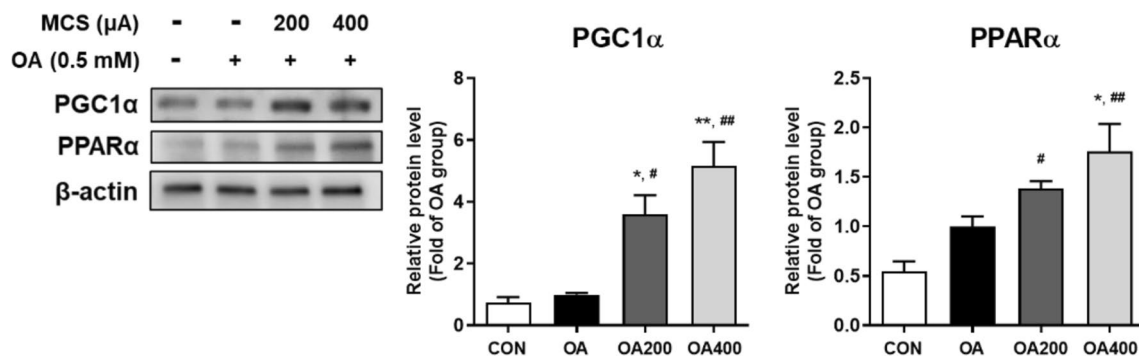


Fig. 17 Immunoblot analysis of the transcription factors regarding fatty acid β -oxidation in FL83B cells [146]

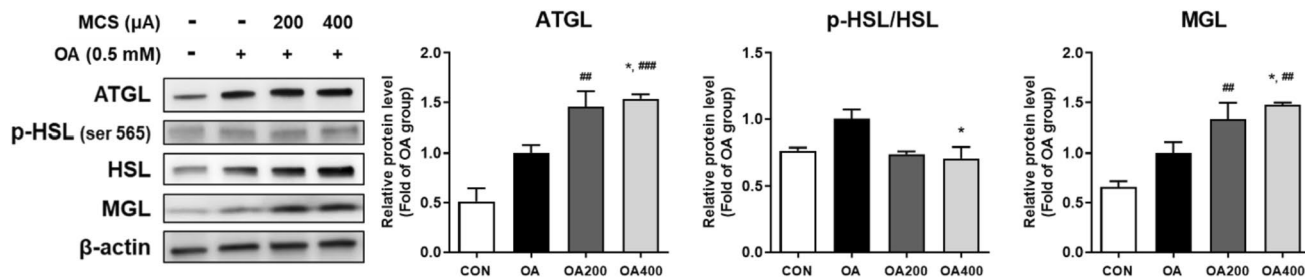


Fig. 18 Immunoblot analysis of the protein expression regarding lipolysis pathway in FL83B cells [146]

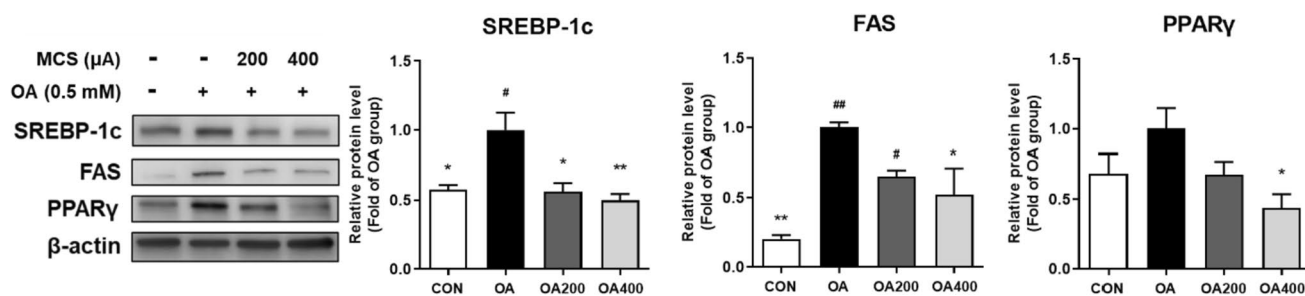
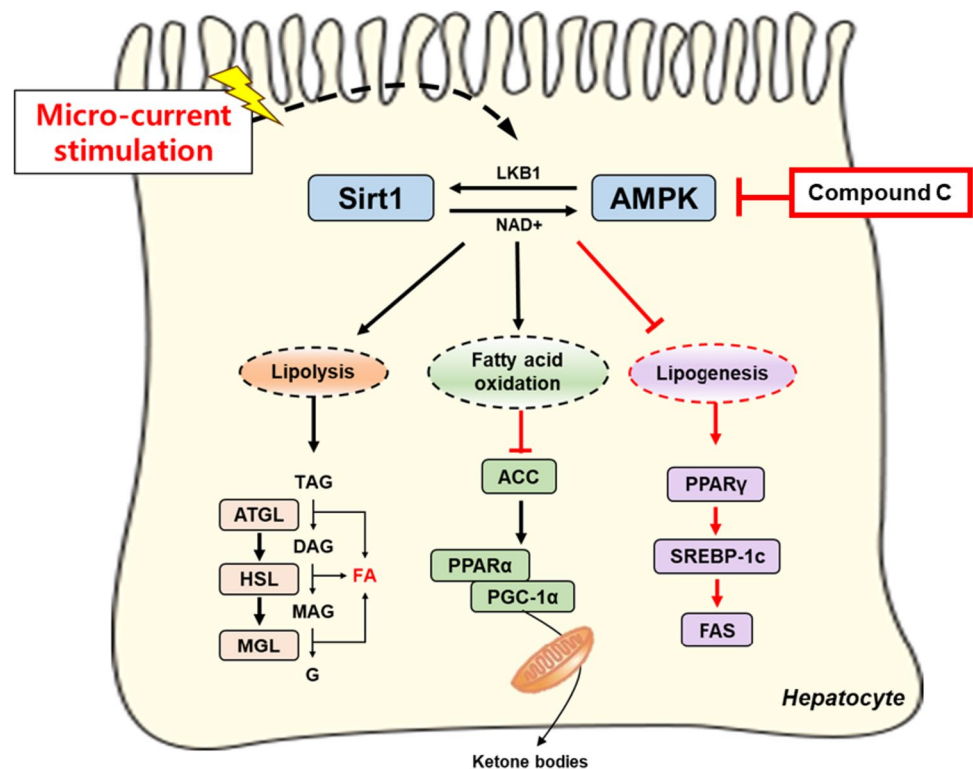


Fig. 19 Immunoblot analysis of the transcription factors regarding lipogenesis in FL83B cells [146]

Fig. 20 Schematic diagram of the regulating lipid metabolism of micro-current stimulation on hepatocytes [146]



activating the Wnt/ β -catenin pathway, which suppresses adipogenic proteins like C/EBP α and lipid accumulation.

There are a growing number of documented scientific therapeutic uses of electricity. In most cases, although the mechanism is not always clearly understood, it appears to arise as a consequence of the depolarization of excitable cell membranes resulting from the applied currents. Other mechanisms appear to be involved in electro-chemical effects. A good deal of evidence regarding the effects of MCS on tissue healing has accumulated over recent decades [53, 179–181]. Where clinical trials have been reported, they are presented, though reference to in vitro and animal studies is also made where clinical trial data is scarce. Therefore, the main focus of this review on those studies that use MCS for the purpose of evaluation in the molecular and cellular levels.

From the overall of this review, we can conclude that MCS appears to initiate a cascade of effects that span from the cellular to the whole-body level. While its applications in various therapeutic contexts are promising, ongoing research aims to deepen our understanding of the specific mechanisms underpinning these effects and refine the optimal use of MCS for therapeutic purposes.

Author contributions H. Lee, S. Cho, and H.S. Kim contributed to the study conception, data collection, and analysis. The first draft was

written by H. Lee and S. Cho and all authors edited and supervised the revisions of the manuscript. All authors read and approved the final manuscript.

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Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

Ethics approval The experiments were approved by the Yonsei University Animal Care Committee (YWCI-202007-013-01, YWCI-201910-016-01, YWCI-201704-007-01) and conducted in accordance with the Guidelines for Animal Experimentation the National Institutes of Health guide for the care and use of Laboratory animals. This information appears in the cited references but is restated here.

Consent to participate Not applicable.

Consent to publish Not applicable.

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